THE RELATIONSHIP BETWEEN METHYL AND TOTAL MERCURY IN ONTARIO FISH

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ABSTRACT

Nearly 600 fish representing 16 species were analyzed for methyl and total mercury. The average methyl to total mercury ratio was 88.9%, indicating that essentially all of the mercury in fish muscle is present as methyl mercury. The effect of age, weight, and length on the methyl/total mercury ratio was examined, and it was found that these variables have no effect on the ratio. The ratio was similar for all sizes, ages, and species, although some very small differences were noted between shallow, warm water fish and bottom feeders, with respect to the ratio. The effect of industrialization on the methyl/total mercury ratio was found to be insignificant.

INTRODUCTION

It has long been assumed, partly on the basis of analytical work performed but unpublished by many laboratories, and partly based on published information, that most of the mercury in fish is present as the methyl mercury form. However, there is not enough evidence to document the actual ratio of methyl to total mercury in a fish, or the variability of this ratio from species to species. Also, the relationship between this ratio and the age, length or weight of various species is not well documented, nor is the effect of industrialization on the ratio.

The Ministry of the Environment, along with various other provincial and federal government groups, is currently involved in large scale analytical programs with respect to the public health significance of mercury. If it could be assumed that virtually all of the mercury in fish is present as the methyl mercury form, then the rapid flameless AAS technique (1) could be used following adequate preparation of fish samples, rather than the lengthy gas chromatographic method of Westoo (2).

The study described herein was designed with the following objectives in mind. First, the methyl mercury to total mercury ratio in fish from the St. Clair System should be established including possible specietal variation. Second, to establish the relationship between the methyl mercury to total mercury ratio and the size or age of certain species. Third, the effect of industrialization on the methyl mercury to total mercury ratio should be examined.

The bulk of the published data concerning the ratio of methyl mercury to total mercury, while usually restricted to one or two species or to specific aspects of the methyl/total mercury ratio, generally indicates that nearly all of the mercury found in the edible portion of fish is in the monomethyl form. Westoo, as early as 1966, had found that "90% of the total mercury was methyl mercury, but about 75% was a more common result" (2). In 1967, Westoo had modified her analytical method, and she reported that 92% of the total mercury in fish muscle was present as methyl mercury (3). Since then, a number of researchers have reported that virtually all of the mercury in fish muscle is present as monomethyl mercury (4-12). Kamps, in the most comprehensive study on the subject to date, reports comparative data from several laboratories based on the analysis of 36 samples, and states "mercury in the edible portion of these swordfish, tuna fish, northern

pike, white bass, and perch is essentially all monomethyl mercury" (13).

Not all workers have demonstrated this relationship, especially some of the earlier Japanese researchers. Lindstedt and Skerfving (5) quote Japanese work showing methyl mercury concentrations ranging from 0 to 75% of the total mercury, with an average of 25%. Kamps et. al. (13) quote others that cite methyl mercury levels comprising 50% of the total mercury in tuna fish. Ueda, Aoki, and Nishimura (14) reported that 4 to 65% of the total mercury in Japanese fish was alkyl mercury.

However, Westoo has produced convincing evidence that the methyl mercury determinations were too low in the methods of some Japanese investigators (15). In a direct comparison between Swedish and Japanese laboratories, the Japanese method produced consistently low recoveries of methyl mercury. Westoo concluded that most of the mercury present in Japanese fish was present in the methyl form, but the analytical methods employed recovered only a fraction of this compound from the fish.

A marine species, Pacific Blue Marlin, was reported to have a methyl to total mercury ratio of about 25% (16), but this data has not been confirmed. The same author provides evidence of methyl-total mercury ratios for several other marine species in excess of 90%.

Bache, Gutenmann, and Lisk (17) have reported that the proportion of methyl mercury to total mercury in lake trout increases with age, ranging from 31% to 102%, however, these analyses were not done on fish muscle, but were done on ground whole fish samples.

Work carried out in our laboratory indicates that both methyl and total mercury levels in some fish specimens are dependent on whether the sample comprises a ground whole fish or a muscle fillet. Furthermore, it has been noted that data obtained from ground whole fish is less precise and consequently these findings lead us to believe that muscle fillet is a better choice for sampling fish that are to be analyzed for mercury.

PROCEDURE

A: Analytical

Approximately 650 fish from the Lake St. Clair System* and 55 fish from Minnitaki Lake ** were captured, quick frozen, and submitted for analysis. After thawing, the fish were sampled to provide about five grams of epaxial musculature.

These samples were analyzed for methyl mercury and total mercury, using gas-liquid chromatographic and flameless atomic absorption spectrophotometric methods (18, 19) which are modifications of procedures published elsewhere (1, 2).

B: Statistical

As previously mentioned, the data used for this study were derived from fish caught in the Lake St. Clair area, and included representatives of 29 species (see Table IV for a complete listing of these species). These fish were examined with respect to the following variables: methyl mercury concentration, total mercury concentration, methyl/total mercury ratio, length, weight, and in two cases where data was available, the age factor was also assessed.

Initially, the sample population consisted of 732 fish representing these 29 species. However, only 16 species were found to be represented by more than 10 specimens, so it was decided to restrict the investigation to these 16 species, amounting to 576 specimens.

In order to evaluate the possible effects of the degree of industrialization on the methyl/total mercury ratio, it was decided to compare the methyl/total mercury ratios of two species from Lake St. Clair to the ratios of the same two species from Minnitaki Lake.

* The "Lake St. Clair System" refers to the St. Clair River and Lake St. Clair, and does not include any of the tributories leading to or from the river or lake.

** Minnitaki Lake is situated in Northwestern Ontario, near latitude 50°, longitude 92^{0}.

A correlation was employed to statistically compare the various specietal parameters, since there were random variations associated with each parameter. Also, it was judged that this type of statistical analysis would demonstrate the associations of the various parameters with a minimum of assumptions regarding their interdependence.

In order that a correlation be valid, the variables must follow a bivariate normal distribution. For this reason, the parameters associated with five of the sixteen species were tested for normality (by methods outlined in Appendix B) and all were found to be normally distributed. On this basis, the other populations were assumed to be normal.

In order to thoroughly evaluate the data generated, it was decided to employ a five variable correlation in which all possible pairs of the five parameters monitored for each fish were correlated with each other. A separate five variable correlation was performed on each of the 16 species for which there was a significant population. The results of these tests are presented as correlation matrices and are included as Tables V-XXII, Appendix A.

From the sixteen five variable correlations, the following relevant data can be obtained: methyl mercury levels, total mercury levels, the methyl mercury to total mercury ratio, the standard deviation and maximum and minimum data for each of these, and the correlation of the ratio with length, weight, and where available, age data. In addition, significance tests were performed on each of the correlation coefficients to determine their validity. Separate five variable correlations were performed on the pike and pickerel data from Lake Minnitaki for purposes of comparison with the corresponding species from Lake St. Clair.

RESULTS

The results of the study are presented in three groups, as they pertain to the three objectives under consideration: the establishment of a methyl mercury to total mercury ratio for freshwater fish in Ontario, the relationship between this ratio and such variables as species, age, length or weight, and finally, the effect of industrialization on the methyl mercury/total mercury ratio.

A: Total and Methyl Mercury Data and Ratios.

Methyl and total mercury data obtained by chemical analysis of the fish specimens from Lake St. Clair were averaged. The methyl/total mercury ratio was obtained by dividing each fish's methyl mercury result by its total mercury result. These ratios were averaged for each species, and this data is presented in Table I.

TABLE I - TOTAL AND METHYL MERCURY, AND RATIO DATA.

Species	n	TOT Hg (ppm)			Methyl Hg (ppm)			Me Hg/TOT Hg Ratio (%)			
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	(RSD)
Pumpkinseed	22	1.10	2.80	1.66	0.97	2.40	1.40	66.7	109.1	84.8	9.8
Bluegill	36	0.58	3.20	1.67	0.49	2.80	1.36	65.2	100.0	81.8	8.7
Perch	65	0.13	3.30	1.09	0.10	3.20	0.98	65.0	111.3	89.0	11.0
Northern Pike	69	1.80	9.90	5.02	1.60	8.95	4.35	59.3	111.4	86.8	11.0
Bowfin	16	0.51	11.6	4.91	0.47	7.6	3.84	64.5	109.5	85.4	14.0
Black Crappie	36	0.79	3.70	2.20	0.64	3.30	1.87	72.4	100.0	85.4	7.9
Largemouth Bass	14	1.04	4.12	3.06	1.07	3.87	2.71	78.0	111.7	90.2	10.0
Northern Redhorse	28	0.07	5.16	1.35	0.07	5.00	1.26	76.2	106.7	92.1	8.0
Yellow Bullhead	27	0.62	4.88	2.18	0.52	4.00	1.88	74.8	104.3	86.9	7.9
Channel Cat	11	1.05	3.94	2.53	0.99	3.55	2.31	77.3	104.5	92.0	7.6
Yellow Walleye	97	0.27	5.80	1.19	0.29	4.50	1.07	62.0	119.6	88.5	12.0
Freshwater Drum	22	0.11	1.89	0.77	0.11	1.68	0.73	77.2	111.1	95.1	7.1
White Bass	27	0.44	3.35	2.10	0.38	3.58	1.96	76.0	112.7	93.2	10.0
Muskellunge	13	1.08	23.29	5.05	0.92	20.41	4.41	75.5	100.0	88.0	6.6
Rock Bass	63	0.22	4.25	1.69	0.16	4.21	1.57	71.6	106.9	92.1	8.2
Carp	30	0.31	2.90	1.49	0.25	2.70	1.40	76.9	117.4	95.3	10.0

B: Relationship Between Methyl/Total Mercury Ratio and Age, Weight or Length (16 Species).

Correlations were performed for all 16 species between the methyl/total mercury ratio and weight, the ratio and length, and for two species, between the ratio and age. These data were obtained from the species correlation matrices, Tables V-XXII, Appendix A, and are shown in Table II.

TABLE II

Species	n	Methyl to TOT Hg	CORRELATION COEFFICIENTS: Me/TOT Hg Ratio to Weight	Me/TOT Hg to Length	Me/TOT Hg to Age
Pumpkinseed	22	0.900*	-0.103	-0.129	NA
Bluegill	36	0.938*	-0.303*	-0.343*	NA
Perch	65	0.991*	0.063	0.224	0.032
Northern Pike	69	0.935*	0.154	0.161	NA
Bowfin	16	0.969*	-0.262	-0.280	NA
Black Crappie	36	0.957*	0.117	0.082	NA
Largemouth Bass	14	0.933*	0.067	0.095	NA
Northern Redhorse	28	0.997*	-0.534*	-0.507*	NA
Yellow Bullhead	27	0.982*	0.101	0.048	NA
Channel Cat	11	0.984*	-0.442	-0.546	NA
Yellow Walleye	97	0.982*	0.043	0.062	0.061
Freshwater Drum	22	0.986*	0.154	0.045	NA
White Bass	27	0.983*	-0.181	-0.103	NA
Muskellunge	13	0.999*	0.063	0.104	NA
Rock Bass	63	0.992*	0.092	0.073	NA
Carp	30	0.958*	-0.02	-0.008	NA

*Denotes that the correlation coefficient is significantly different from a random correlation, to a 95% level of probability.

C: Effect of Industrialization on Methyl/Total Mercury Ratio.

Two species of fish from Lake St. Clair were compared to representatives of the same species in Minnitaki Lake with respect to methyl/total mercury content. These results, as well as data regarding total and methyl mercury content, and the correlation between the methyl/total mercury ratio and weight and length are presented in Table III.

TABLE III: COMPARISON OF FISH FROM MINNITAKI LAKE AND LAKE ST. CLAIR.

Parameter	Minnitaki Lake		Lake St. Clair	
	Walleye	Northern Pike	Walleye	Northern Pike
Total Mercury (Min)	0.30	0.25	0.27	1.80
Total Mercury (Max)	1.31	2.00	5.80	9.90
Total Mercury (Mean)	0.69	0.71	1.19	5.02
Methyl Mercury (Min)	0.26	0.24	0.29	8.60
Methyl Mercury (Max)	1.30	1.63	4.50	8.95
Methyl Mercury (Mean)	0.61	0.62	1.07	4.35
Methyl/Total Hg Ratio (Min)	63.4	68.0	62.0	59.3
Methyl/Total Hg Ratio (Max)	119.4	102.6	119.6	111.4
Methyl/Total Hg Ratio (Mean)	87.9	87.2	88.5	86.8
S.D. (Me/TOT Hg Ratio)	13.4	8.3	11.8	10.8
N	23	31	97	69

DISCUSSION

The study produced methyl and total mercury results for over 700 fish representing 29 species. Because some species were only poorly represented, 16 species (576 fish from Lake St. Clair and 54 from Minnitaki Lake) were considered for the purposes of this report.

The methyl/total mercury ratio has been established for all 16 species, and an average ratio is presented; the relationship between the methyl/total mercury ratio and such variables as species, age, length and weight has been investigated; and the effect of industrialization on the methyl/total mercury ratio has been examined.

A: Methyl/Total Mercury Ratio.

The results of this investigation support the conclusions of other researchers (2-13) that essentially all of the mercury present in fish muscle exists as the methyl mercury form. Methyl/total mercury ratios were derived for 16 species of fish, as shown in Table I. The mean ratio for these species ranges from a low of 81.8% for Bluegill to a high of 95.3% for Carp.

It is understood that these values are highly dependent upon analytical sensitivity, precision, and accuracy. Accordingly, the analytical methods employed were stringently controlled through daily standardization and calibration of both the gas chromatograph and the flameless AAS procedures. In addition, "control" fish, whose mercury content had been determined by at least fifty prior determinations, were analyzed daily by both procedures. Recovery values were established for the methods, by spiking fish with known amounts of mercuric chloride and/or methyl mercury chloride or bromide. Furthermore, a number of round robin fish samples received during the interval of this study were analyzed as part of our regular participation in such collaborative tests. When adequate sample was available, replicate analyses were performed in order to establish precision.

Daily analysis of the "control" fish for total and methyl mercury by flameless AAS and gas chromatography yielded precision values, in terms of relative standard deviation, of $\pm$ 6% and $\pm$ 7% respectively across the range 0.2 to 2.1 ppm. Replicate analysis of a large (Smallmouth Bass) fish as one run indicated a relative S.D. of

0 $\pm$ 5.2% at the 1.8 ppm level.

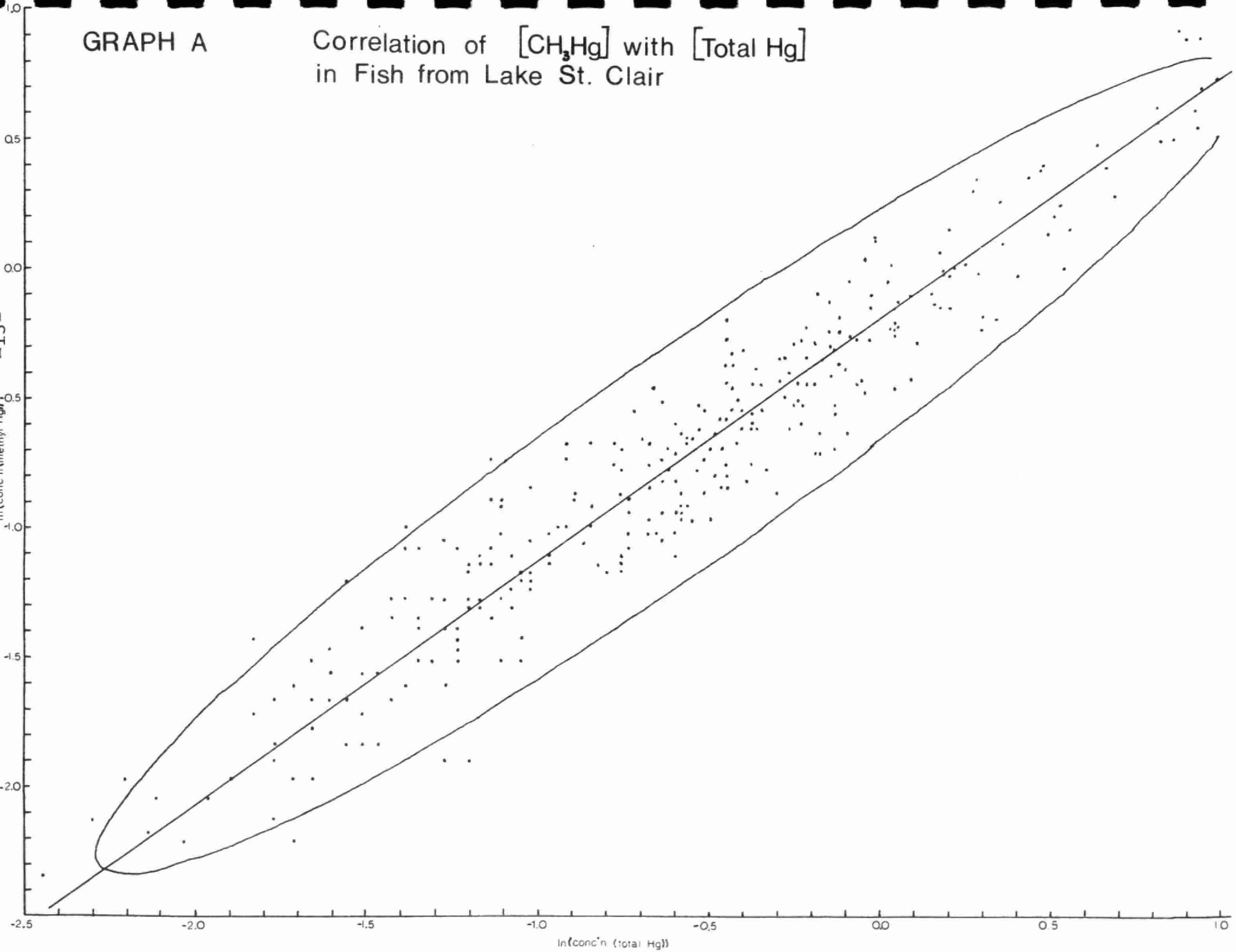
Accuracy data, obtained from the round robin studies, indicate an accuracy of 95% for total mercury and 85% for methyl mercury analyses. These data were obtained by assuming the mean of all participating laboratories' data to be the true value, dividing our result by this mean and multiplying by 100 to convert to percent. Fifteen round robin samples received between December 1972 and February 1973 were used for this calculation; the accuracy figures quoted are mean values.

Recovery studies were also performed and the following results were obtained: for methyl mercury analysis, the recovery of methyl mercuric bromide added to fish tissue was 89.5 $\pm$ 4.2%. For the total mercury procedure, the recovery of methyl mercuric chloride was 95.0 $\pm$ 3% and for mercuric chloride it was 94.3 $\pm$ 2%.

The methyl/total mercury ratio, calculated as a mean of all 576 fish from Lake St. Clair, was found to be 88.9%. This ratio is consistent over the entire range of methyl and total mercury concentration levels, and this is supported by the high correlation coefficients shown in Table II.

These correlation coefficients indicate that for the 16 species examined, there is an almost directly proportional relationship between methyl mercury content and total mercury content. The coefficients range from 0.900 to 0.999, and are significantly greater than zero; that is, there is less than one chance in 1,000 that these coefficients are the result of a random relationship. This further substantiates the findings of other researchers (2-13) and leads to the conclusion that essentially all of the mercury present in the sixteen species studied is in the methyl mercury form.

Graph A illustrates the correlation of methyl to total mercury irrespective of species, for Lake St. Clair.



GRAPH A Correlation of [CH_3Hg] with [Total Hg] in Fish from Lake St. Clair

B: Relationships Between Methyl/Total Ratio and Age, Weight, Length and Species.

Even though the correlation between methyl and total mercury was strong, and the methyl/total mercury ratios were in the order anticipated, it was deemed desirable that the possible dependence of the ratio upon certain variables should be investigated.

The work of Bache, Gutenmann and Lisk (17) indicated that there exists a significant relationship between the age of a fish and the methyl/total ratio. This finding prompted an investigation on our part of such a relationship, for the two species for which we had age data, namely Perch and Yellow Walleye. It was found that while the methyl/total mercury ratios were typical, that is 89% and 88.5% for Perch and Walleye respectively, there was no significant relationship between the ratio and the age of the fish, as indicated by the very low correlation coefficients shown in Table II.

While age data was not available for the rest of the species, weight and length data was available for all 16 species.

Table II lists correlation coefficients which indicate that there is no significant relationship between the methyl/total mercury ratio and length or between the ratio and weight, except for Bluegill and Northern Red-horse. For these two species, a negative relationship is suggested, but the coefficients are too low to be useful in drawing conclusions.

While our data refutes the conclusions of Bache, Gutenmann and Lisk (17), it must be pointed out that their data base consisted of analyses performed on whole fish, while our data is based entirely on fillet analysis. As earlier mentioned, it had been noted in this laboratory that large discrepancies can arise between methyl mercury and total mercury results, and between the methyl/total mercury ratios on the same fish, depending on whether the results are obtained by analyzing whole fish or fillets from the fish. It has not yet been established whether these differences are due to sample heterogeniety or alterations in the extraction efficiencies of the methyl mercury procedure. Nonetheless, the data presented here establishes that the methyl/total mercury ratio is essentially invariable with age for the species studied.

Apparently, the methyl/total mercury ratio varies somewhat among species. However, because of analytical variation and the standard deviations associated with the ratios, it is difficult to ascertain whether these differences are significant. Nevertheless, there does appear to be some association between the magnitude of the ratio for certain species, and the depth at which they most frequently feed. The species of fish that are generally among the highest in terms of methyl/total mercury ratios are benthic feeders, such as Freshwater Drum, Redhorse Sucker, Carp and Channel Cat, while the species with the lowest methyl/total mercury ratio are Bluegill and Pumpkinseed, which are shallow, warm water fish.

Regarding the average total mercury levels in the various species examined, there is the expected progression from smaller species to the larger, more predacious ones (Muskellunge and Northern Pike both have mean values close to 5 ppm). Thus, significant species differences exist for total and for methyl mercury content.

C: Effect of Industrialization on Methyl/Total Mercury Ratio.

Adult fish from Minnitaki Lake were compared to the same species in Lake St. Clair, with respect to methyl mercury and total mercury content and methyl/total mercury ratio. Minnitaki Lake was selected because it was considered to have little or no direct input of mercury due to industrial or agricultural activity. The lake is located in Northwestern Ontario, close to Sioux Lookout, and has no industry or any known mercury-containing effluents on its shores. The fish examined in this study were of two species, Yellow Walleye and Northern Pike, and represented a whole range of ages. Average sizes for species between the two lakes were similar.

The methyl mercury and total mercury content in Lake Minnitaki fish is considerably lower than in Lake St. Clair fish (see Table III). For example, Yellow Walleye from Minnitaki Lake had an average value of 0.69 ppm total mercury and 0.61 ppm methyl mercury, whereas the same species in Lake St. Clair averaged 1.19 ppm total mercury and 1.07 ppm methyl mercury. The difference between the two lakes for Northern Pike was even greater, since the average values in Lake Minnitaki were 0.71 ppm total mercury and 0.62 ppm methyl mercury, and in Lake

St. Clair they were more than five times higher: 5.02 ppm total mercury and 4.35 ppm methyl mercury.

On the other hand, the mean methyl/total mercury ratios are almost identical, not only for both species of fish, but for both species in both lakes. The actual mean ratios are: Walleye, Minnitaki Lake: 87.9%; Walleye, Lake St. Clair: 88.5%, and for Northern Pike, Minnitaki Lake: 87.2%, Northern Pike, Lake St. Clair: 86.8%. These ratios are so similar that it appears extremely unlikely that the different degree of industrialization in these two water bodies has any effect upon the methyl to total mercury ratio.

It is not the purpose of this report to attempt a classification of areas according to mercury inputs, but these two water bodies afforded an opportunity to examine the effects of known massive mercury contamination, relative to an area not known to have suffered significant industrially-transported mercury contamination. Some sediments in Lake St. Clair contain quite high levels of mercury, and very high levels have been found in sediments of the St. Clair River. Lake Minnitaki, however, has no known influx of mercury, but could have been subject to occasional atmospheric fallout of mercury. This does not seem to be likely, but even if it were the effect on the fish in Minnitaki seems slight, since the levels of mercury encountered in the Minnitaki fish are in the same order as those associated with adult specimens of the same species in unpolluted lakes.

The effect of known mercury contamination on the two species examined is extreme in terms of total and methyl mercury content, while the effect on the methyl/ total ratio is insignificant.

In order to strengthen the conclusion that industrialization has an insignificant effect on the methyl/total mercury ratio, a fish donated by the Royal Ontario Museum was analyzed for methyl and total mercury content. The fish was a Muskellunge, captured in 1951 at Nogies Creek near its outflow to Buckhorn Lake (near Peterborough, Ontario) and the ratio, established by replicate methyl and total mercury analyses, was found to be 85.4%. This corroborates the other data and strengthens the conclusion that the methyl/total mercury ratio for the species examined is not likely to vary much from the mean value of 88.9%.

SUMMARY AND CONCLUSIONS

A series of 576 fish, representing 16 species, were analyzed for methyl and total mercury. Methyl/total mercury ratios were calculated for all 16 species, and found to range from 81.8% to 95.3% with an overall mean ratio of 88.9%. The interrelationships of methyl mercury concentration, total mercury concentration, methyl/total mercury ratio, weight, length and where available, age were investigated. It was determined that methyl and total mercury were closely related, with correlation coefficients ranging from 0.900 to 0.999.

The relationships between the methyl/total mercury ratio and age, weight and length were examined and the ratio was found to be constant irrespective of age or size. However, certain trends were observed between the methyl/total mercury ratio and the feeding habits of some species. The effect of industrialization on the methyl/total mercury ratio was examined, and it was established that no effect on the ratio could be attributed to industrialization in the areas studied.

The following conclusions were drawn from this study:
1. essentially all of the mercury found in fish muscle is present as the methyl mercury form.

2. the ratio of methyl/total mercury in the fillet is not dependent upon the age, weight or length of the fish.

3. presence of a direct industrial input of mercury in the areas examined did not affect the methyl/total mercury ratio to any degree.

TABLE IV

Species of fish analyzed:

Golden Shiner	(Notemigomus chrysolucas)
Blackchin Shiner	(Notropis heterodon)
Emerald Shiner	(N. atherinoides)
Spottail Shiner	(N. hudsonis)
Blacknose Shiner	(N. heterolepis)
Gizzard Shad	(Dorosoma cepedianum)
Goldfish	(Carassius auratus)
Pumpinseed	(Lepomis gibbosus)
Bluegill	(L. macrochirus)
Brook Silversides	(Labidesthes sicculus)
Yellow Perch	(Perca flavescens)
Bowfin	(Amia calva)
Black Crappie	(Pomoxis nigromaculatus)
White Crappie	(P. annularis)
Largemouth Bass	(Micropterus salmoides)
Smallmouth Bass	(M. dolomieui)
Carp	(Cyprinus carpio)
Northern Redhorse	(Moxostoma macrolepidotum)
White Sucker	(Catostomus commersoni)
Yellow Bullhead	(Ictalurus natalis)
Brown Bullhead	(I. nebulosus)
Channel Cat	(I. punctatus)
Yellow Walleye	(Stizostedion vitreum)
Spotted Sucker	(Minytrema melanops)
Fresh Water Drum	(Aplodinotus grunniens)
White Bass	(Roccus chrysops)
Gar Pike	(Lepisosteus ossius)
Rock Bass	(Amblopites rupestris)
Muskellunge	(Esox masquinongy)

APPENDIX A

TABLE V

CORRELATION MATRIX

SPECIES: Pumpkinseed N: 22

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.254	-0.177	-0.103	-0.129
Methyl Hg	0.254	1.000*	0.900*	0.151	0.204
Total Hg	-0.177	0.900*	1.000*	0.227	0.288
Weight	-0.103	0.151	0.227	1.000*	0.985*
Length	-0.129	0.204	0.288	0.985*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE VI

CORRELATION MATRIX

SPECIES: Bluegill N:36

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.337*	0.009	-0.303	-0.343*
Methyl Hg	0.337*	1.000*	0.938*	0.363*	0.291
Total Hg	0.009	0.938*	1.000*	0.492*	0.430*
Weight	-0.303	0.363*	0.492*	1.000*	0.962*
Length	-0.343*	0.291	0.430*	0.962*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE VII

CORRELATION MATRIX

SPECIES: Yellow Perch N: 65

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length	Age
Me/Tot Hg	1.000*	0.324*	0.218	0.056	0.224	0.032
Methyl/Hg	0.324*	1.000*	0.991*	0.611*	0.620*	0.564*
Total Hg	0.218	0.991*	1.000*	0.641*	0.641*	0.584*
Weight	0.056	0.611*	0.641*	1.000*	0.899*	0.522*
Length	0.224	0.620*	0.641*	0.899*	1.000*	0.567*
Age	0.032	0.564*	0.584*	0.522*	0.567*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE VIII

CORRELATION MATRIX

SPECIES: Northern Pike N: 69

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.318	-0.030	0.154	0.161
Methyl Hg	0.318	1.000*	0.935*	0.255*	0.294*
Total Hg	-0.030	0.935*	1.000*	0.208	0.253
Weight	0.154	0.255*	0.208	1.000*	0.939*
Length	0.161	0.294*	0.253*	0.939*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE IX

CORRELATION MATRIX

SPECIES: Bowfin N: 16

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.576*	-0.734*	-0.262	-0.280
Methyl Hg	-0.576*	1.000*	0.969*	0.232	0.252
Total Hg	-0.734*	0.969*	1.000*	0.243	0.272
Weight	-0.262	0.232	0.243	1.000*	0.973*
Length	-0.280	0.252	0.272	0.973*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE X

CORRELATION MATRIX

SPECIES: Black Crappie N: 36

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.224	-0.055	0.117	0.082
Methyl Hg	0.224	1.000*	0.957*	0.786*	0.776*
Total Hg	-0.055	0.957*	1.000*	0.762*	0.766*
Weight	0.117	0.786*	0.762*	1.000*	0.969*
Length	0.082	0.776*	0.766*	0.969*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XI

CORRELATION MATRIX

SPECIES: Large Mouth Bass N: 14

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.119	-0.462	0.067	0.095
Methyl Hg	-0.119	1.000*	0.933*	0.059	0.199
Total Hg	-0.462	0.933*	1.000*	0.013	0.129
Weight	-0.067	0.059	0.013	1.000*	0.971*
Length	0.095	0.199	0.129	0.971*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XII

CORRELATION MATRIX

SPECIES: Northern Redhorse N: 28

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.240	0.175	-0.534*	-0.507*
Methyl Hg	0.240	1.000*	0.997*	0.097	0.224
Total Hg	0.175	0.997*	1.000*	0.144	0.270
Weight	-0.534*	0.097	0.144	1.000*	0.963*
Length	-0.507*	0.224	0.270	0.963*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XIII

CORRELATION MATRIX

SPECIES: Yellow Bullhead N: 27

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.009	-0.166	0.101	0.048
Methyl Hg	-0.009	1.000*	0.982*	0.354	0.057
Total Hg	-0.166	0.982*	1.000*	0.341	0.063
Weight	0.101	0.354	0.341	1.000*	0.857*
Length	0.048	0.057	0.063	0.857*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XIV

CORRELATION MATRIX

SPECIES: Channel Catfish N: 11

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.193	-0.360	-0.442	-0.546
Methyl Hg	-0.193	1.000*	0.984	0.306	0.481
Total Hg	-0.360	0.984*	1.000*	0.365	0.538
Weight	-0.442	0.306	0.365	1.000*	0.825*
Length	-0.546	0.481	0.538	0.825*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XV

CORRELATION MATRIX

SPECIES: Walleye N: 97

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length	Age
Me/Tot Hg	1.000*	0.256*	0.104	0.043	0.062	0.061
Methyl Hg	0.256*	1.000*	0.982*	0.717*	0.707*	0.728*
Total Hg	0.104	0.982*	1.000*	0.729*	0.712*	0.736*
Weight	0.043	0.717*	0.729*	1.000*	0.903*	0.958*
Length	0.062	0.707*	0.712*	0.903*	1.000*	0.913*
Age	0.061	0.728*	0.736*	0.958*	0.913*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XVI

CORRELATION MATRIX

SPECIES: Fresh Water Drum N: 22

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.110	-0.265	0.154	0.045
Methyl Hg	-0.110	1.000*	0.986*	0.404	0.541*
Total Hg	-0.265	0.986*	1.000*	0.387	0.536*
Weight	0.154	0.404	0.387	1.000*	0.974*
Length	0.045	0.541*	0.536	0.974*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XVII

CORRELATION MATRIX

SPECIES: White Bass N: 27

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.206	0.049	-0.181	-0.103
Methyl Hg	0.206	1.000*	0.983*	0.499*	0.586*
Total Hg	0.049	0.983*	1.000*	0.547*	0.622*
Weight	-0.181	0.499*	0.547*	1.000*	0.948*
Length	-0.103	0.586*	0.622*	0.948*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XVIII

CORRELATION MATRIX

SPECIES: Muskellunge N: 13

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.039	-0.085	0.063	0.104
Methyl Hg	-0.039	1.000*	0.999*	0.717*	0.676*
Total Hg	-0.085	0.999*	1.000*	0.710*	0.670*
Weight	0.063	0.717*	0.710*	1.000*	0.916*
Length	0.104	0.676*	0.670*	0.916*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XIX

CORRELATION MATRIX

SPECIES: Rock Bass N: 63

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.335*	0.224	0.092	0.073
Methyl Hg	0.335*	1.000*	0.992*	0.756*	0.740*
Total Hg	0.224	0.992*	1.000*	0.776*	0.763*
Weight	0.092	0.756*	0.776*	1.000*	0.980*
Length	0.073	0.740*	0.763*	0.980*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XX

CORRELATION MATRIX

SPECIES: Carp N: 30

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.115	-0.370	-0.026	-0.008
Methyl Hg	-0.115	1.000*	0.958*	0.097	0.146
Total Hg	-0.370	0.958*	1.000*	0.063	0.099
Weight	-0.026	0.097	0.063	1.000*	0.961*
Length	-0.008	0.146	0.099	0.961*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XXI

CORRELATION MATRIX

SPECIES: Walleye (Minnitaki) N: 23

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	0.181	-0.147	0.205	0.275
Methyl Hg	0.181	1.000*	0.942*	0.758*	0.735*
Total Hg	-0.147	0.942*	1.000*	0.728*	0.683*
Weight	0.205	0.758*	0.728*	1.000*	0.980*
Length	0.275	0.735*	0.683*	0.980*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

TABLE XXII

CORRELATION MATRIX

SPECIES: Northern Pike (Minnitaki) N: 31

	Me/Tot Hg	Methyl Hg	Total Hg	Weight	Length
Me/Tot Hg	1.000*	-0.126	-0.295	-0.212	-0.244
Methyl Hg	-0.126	1.000*	0.981*	0.601*	0.645*
Total Hg	-0.295	0.981*	1.000*	0.606*	0.657*
Weight	-0.212	0.601*	0.606*	1.000*	0.955*
Length	-0.244	0.645*	0.657	0.955*	1.000*

* Indicates a correlation coefficient significantly different from zero at the 95% level of probability.

A P P E N D I X B

APPENDIX B.

Statistical Formulae.

(i) Correlation.

The calculated correlation coefficient was the product-moment correlation coefficient, and is defined

$$r_{xy} = \frac{\sum_{i=1}^{N} x_i y_i}{\sqrt{\sum_{i=1}^{N}(x_i)^2 \sum_{i=1}^{N}(y_i)^2}} \qquad (20)$$

The 95% correlation ellipse has axes in the ratio λ_1/λ_2, where

$$\lambda_1 = \frac{S_x^2 + S_y^2 + D}{2} \qquad \lambda_2 = \frac{S_x^2 + S_y^2 - D}{2}$$

$$D = \sqrt{(S_x^2 + S_y^2)^2 - 4(S_x^2 S_y^2 - S_{xy}^2)} \qquad (21)$$

$$S_x^2 = \frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{(N-1)} \qquad S_y^2 = \frac{\sum_{i=1}^{N}(y_i - \bar{y})^2}{(N-1)}$$

$$S_{xy}^2 = \frac{2}{(N-1)} \sum_{i=1}^{N}(x_i - \bar{x})(y_i - \bar{y})$$

The major axis of correlation was determined by the method of least squares.

(ii) Population Normalization.

A data population can deviate from normality by being skewed or kurtic (see Fig. 1 for pictorial representations of skewed

APPENDIX B Cont.

and kurtic populations). To ensure that the correlations were valid, i.e. that the data followed a bivariate normal distribution, the populations on each correlation axis were tested for skewness (g_1) and kurtosis (g_2). The relevant formulae are

$$g_1 = \left[\frac{\dfrac{\sum_{i=1}^{N}(w_i - \bar{w})^3}{(N-1)}}{\left(\sqrt{\dfrac{\sum_{i=1}^{N}(w_i - \bar{w})^2}{(N-1)}}\right)^3} \right]$$

$$\tag{22}$$

$$g_2 = \left[\left\{ \frac{\dfrac{\sum_{i=1}^{N}(w_i - \bar{w})^4}{(N-1)}}{\left(\sqrt{\dfrac{\sum_{i=1}^{N}(w_i - \bar{w})}{(N - 1)}}\right)^4} \right\} - 3 \right]$$

APPENDIX B Cont.

For normal populations, these quantities should be zero. The g_1 and g_2 values for the five correlations are listed in Table XXIII. All values were tested and were found not to be different to zero at the 95% probability level.

TABLE XXIII

Skewness and Kurtosis values for the Correlation Populations.

Correlation	Axis	g_1	g_2
A	x y	0.019 0.035	-0.391 -0.245
B	x y	0.580 0.467	-0.024 0.233
C	x y	0.538 -0.065	-0.578 -0.532
D	x y	-0.014 0.298	-0.828 0.027
E	x y	0.111 0.548	0.503 -0.200

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